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Stable Carbon and Nitrogen Isotopic Composition of Diet and Hair of Gidra-Speaking Papuans

JUN YOSHINAGA, MASAO MINAGAWA, TSUGUYOSHI SUZUKI, RYUTARO OHTSUKA, TOSHIO KAWABE, TSUKASA INAOKA, AND TOMOYA AKIMICHI

Department of Human Ecology, School of Health Sciences, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan (J.Y., T.S., R.O., T.K.); National Institute for Environmental Studies, Ibaraki 305, Japan (J.Y., T.S.); Mitsubishi-kasei Institute of Life Sciences, Tokyo 194, Japan, and Graduate School of Environmental Earth Sciences, Hokkaido University, Sapporo 060, Japan (M.M.); Department of Public Health, Faculty of Medicine, Kumamoto University, Kumamoto 860, Japan (T.I.); National Museum of Ethnology, Osaka 565, Japan (T.A.)

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ABSTRACT The carbon and nitrogen stable isotopic composition of the scalp hair and diet of Gidra-speaking people in four villages in Papua New Guinea is presented. The isotopic composition of hair was measured, while that of the diet was estimated from food consumption survey data and the measured isotopic composition and protein and carbohydrate contents of food items. The average isotopic ratios of the hair samples and of the diet varied among the four study villages, which were selected because of their diverse ecological settings. Comparison was made between hair and calculated dietary isotopic compositions. Two of the four diet-hair enrichment values obtained for ^{13}C (+1.8 and 2.2‰) were similar to those previously reported (1.4–2.0‰), but the other two values (3.7 and 4.8‰) were greater than in earlier reports. ^{15}N enrichment was systematically greater (by 1‰) than reported values (~4.3‰) except for one village, where a much greater enrichment (6.9‰) was found. The factors potentially relevant to these deviations are discussed. Possible errors in estimating the dietary isotopic composition and minor modifications of dietary habits revealed by food consumption surveys could explain most of the discrepancies. However, the great enrichment of ^{15}N found in one of the villages remains unexplained.

Stable carbon and nitrogen isotopic analysis of excavated human bone collagen has become a method for obtaining quantitative information on past diets—for example, relative dependence on C_3 and C_4 plants and on marine and terrestrial products (van der Merwe and Vogel, 1978; Bender et al., 1981; Tauber, 1981; Chisholm et al., 1982). No other methods can provide such information. However, the validity of the past dietary composition reconstructed from isotopic data has not been fully evaluated because of the lack of alternative methods for confirming past dietary composition. One possible and promising approach to the validation of isotopic dietary analysis is to analyze tissue samples from a contemporary human population and compare the dietary composition revealed by isotopic analysis and other method(s).

Hair may be the most suitable tissue in this regard; it is a tissue which can be ob-

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Address reprint requests to J. Yoshinaga, National Institute for Environmental Studies, Onogawa 16-2, Tsukuba-shi, Ibaraki 305, Japan.

tained noninvasively from a living subject. It grows at a relatively constant rate (1 cm/month), and, therefore, average dietary information over several months to a year may be obtained if a suitable length of hair is analyzed.

Schoeller et al. (1986), Minagawa et al. (1986), and Minagawa (1992) revealed that the carbon and nitrogen isotopic composition of human hair from contemporary populations of the USA, Japan, and other modernized countries was consistent with that of diet when isotope fractionation between diet and hair was considered. In these studies, the dietary information was based on the national food consumption statistics of the respective countries. However, the conformity of the dietary habits of the subjects of the sample population (sample size: 5–42) to the national average statistics was not evaluated. In addition, it was highly probable that the isotopic composition of the foods available to the subjects within the sample population was variable, because, in these modernized countries, foods might be transferred or imported from other areas or countries where the isotopic composition of the soil was variable. These factors potentially obscure the isotopic relationship between diet and hair. Therefore, it is necessary to confirm the relationship within a single feeding ecosystem.

We have been studying the human ecology of the Gidra-speaking people in Papua New Guinea since 1970 (Ohtsuka, 1983; Ohtsuka and Suzuki, 1990). The Gidra subsist on sago exploitation, horticulture, fishing/hunting, and the gathering of wild plants; there is minimal introduction of foreign foods. One of the foci of this ongoing project is the examination of the relationship between ecological setting and food and nutrition. For this purpose four villages with diverse ecological settings were selected from the 13 villages scattered in the Gidra's territory (4,000 km^2 (Fig. 1), and intensive food consumption surveys were carried out in 1980–1982 (Ohtsuka et al., 1985). Seventy-eight food samples were collected during this period and analyzed for major nutrients (Ohtsuka et al., 1984) and carbon and nitrogen stable isotopic composition (Yoshinaga et al., 1991). The surveys revealed that dietary composition varied among the villages. The major findings which may be relevant to the isotopic study can be summarized as follows: the diet of the Gidra is composed of a mixture of terrestrial and aquatic (both freshwater and marine) animals, the relative significance of which varied markedly between the villages, and of C_3 plant foods which varied little from village to village.

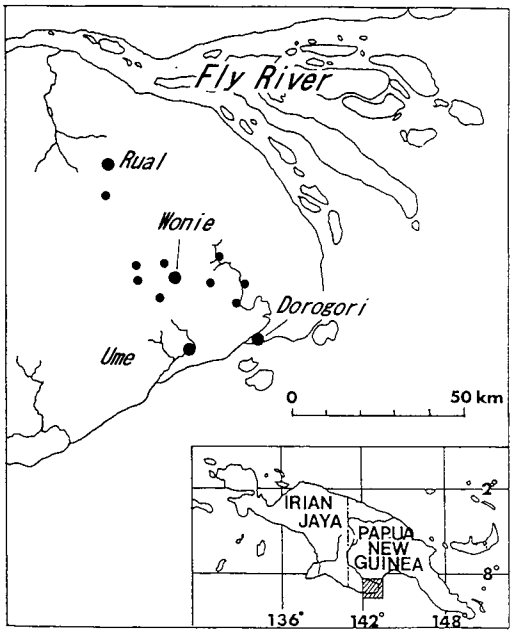

Fig. 1. Map of the Gidra land with names of four study villages.

During the 1980–1982 survey, hair samples were also collected. Thus, the Gidra offer a suitable opportunity to test the validity of dietary analysis based on the stable isotope composition of human tissue. The present paper aims at comparing the isotopic composition of hair and diet of the Gidra. There are four possible advantages of this study over previous works dealing with the diet-hair relationship: 1) hair and foods are from a single ecosystem; 2) intensive food consumption survey data are available; 3) the food composition, including the carbon and nitrogen stable isotopic composition, of the animals and plants that the Gidra actually eat has been determined; and 4) the dietary composition is diverse among the villages.

MATERIALS AND METHODS

Materials

The Gidra inhabit the lowlands of Papua New Guinea. Hair samples were collected from the four study villages of the Gidra in a human ecology survey conducted in 1980–1982. These four villages are Rual (located near a creek in the northern part of the Gidra's territory), Wonie (an inland village located in savanna grassland), Ume (located near a large river), and Dorogori (a coastal village facing an island where the capital town of the Western Province is located) (Fig. 1).

Hair was obtained during personal hairdressing. Sampling was done by the villagers themselves with stainless-steel scissors during the dry season of 1981 (December to May). No instructions for sampling were given; the villagers cut their hair as long as they chose from the tip (distal end sampling) and put it individually into a polyethylene bag. Because of the distal end sampling procedure and the fact that the lengths of the hair samples were not controlled, the period of the formation of the sample material could not be specified and was assumed to vary from subject to subject. Forty-nine samples from males of three age grades of the Gidra, viz., *Miid* (estimated age: >50 years), *Nanyuruga* (40–50 years), and *Rugajog* (20–40 years), from the four study villages were selected for stable isotope analysis.

The nitrogen stable isotope ratios of six food samples of plant origin, which were not included in our previous study, were measured. These six samples were selected because of their abundance in the Gidra's diet. They included sweet potato, elephant-foot yam, banana, lotus seed, cycad seed, and jointfir leaf.

Analytical methods

Carbon and nitrogen in a sample was converted to CO_2 and N_2 by the combustion method (Minagawa et al., 1984). Ten to 15 milligrams of hair sample was used. The N_2 from plant foods was obtained by combusting a 50–120 mg sample. The CO_2 and N_2 obtained were purified in a vacuum line using liquid nitrogen and dry ice–ethanol traps. Stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were determined by a dual-inlet-isotope-ratio-measurement mass spectrometer (Finnigan MAT 251), and they were expressed as $\delta\ ^{13}C$ and $\delta\ ^{15}N$ against international standards (PDB for carbon and atmospheric N_2 for nitrogen) by the equation

$$\delta = \{(R_{sample}/R_{standard}) - 1\} \times 1{,}000 \text{ (unit ‰)}$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The precision of the present isotope ratio measurement was evaluated using histidine as a substandard, and <0.1‰ was obtained for both $\delta\ ^{13}C$ and $\delta\ ^{15}N$ measurements.

Effect of washing method on $\delta\ ^{13}C$ and $\delta\ ^{15}N$ of hair

Hair is, by its very nature, subject to external contamination such as dust, sweat, and skin secretions. Therefore, the effect of washing methods on the isotope ratio was examined using six hair samples from males of Wonie. Approximately 1 g of hair sample was cut with stainless-steel scissors into fine pieces to ensure a homogeneous stock from which subsamples were taken for subsequent washing. Washing methods tested included 1) washing with nonionic detergent (0.4‰ polyoxyethylenelaurylether), 2) washing with organic solvent (chloroform + methanol (2 + 1) and acetone), 3) washing with detergent and solvent, and 4) no washing.

Calculation of dietary isotopic composition based on survey data

Food consumption surveys were carried out in the four study villages during the dry season of 1981. The survey protocol was described in detail by Ohtsuka et al. (1985). Briefly, the weight of food items consumed by the villagers was either measured at the time when they were consumed or estimated by a comparison of input and output of food in subject households during a 14 day survey period, or both. When the consumption unit was a household, adult male daily consumption of each food item was calculated by making appropriate allowance for women and children in the household. This allowance was based on the energy requirement and safe level of protein intake per body weight by sex and age recommended by FAO/WHO

and body weight of the subjects. Details of the conversion process used to make such allowance appeared in Ohtsuka et al. (1985).

The main food items of the Gidra people were sampled in this survey as well as in the 1986 and 1989 surveys, and the nutrient composition of these samples was measured in our laboratory (Ohtsuka et al., 1984). The $\delta\ ^{13}C$ and $\delta\ ^{15}N$ of these samples were also measured and have been reported elsewhere (Yoshinaga et al., 1991). From the data of 1) the food consumption survey, 2) the protein content of each food item, and 3) the $\delta\ ^{13}C$ and $\delta\ ^{15}N$ of each food item, the isotopic composition of daily total ingested protein of an adult male was calculated by the equation

$$\delta_{cal} = (\delta_1 \times w_1 \times p_1 + \delta_2 \times w_2 \times p_2 + \ldots\ldots + \delta_n \times w_n \times p_n)/100/P \quad (1)$$

where δ_{cal} = calculated δ value of diet, δ_n = δ value of food item n, w_n = weight (grams/day/adult male) of the consumption of food item n, p_n = protein content of food item n (grams/100 g), and P = total protein intake (grams/day/adult male).

Since hair is made of keratin, a fibrous protein or group of proteins (Jarrett, 1973), hair carbon and nitrogen isotopic values are thought to represent those of the protein fraction of foods. We had to estimate the δ value of the protein fraction for δ_n in the above equation since δ values of the foods were measured for the carbohydrate + protein fraction (Yoshinaga et al., 1991). We estimated δ_n by calculation according to Nakamura et al. (1982) for the plant food items with the equation

$$\delta\ ^{13}C_n = \delta\ ^{13}C_{total} - 1.5 \times 0.44 \times w_p/(0.44w_c + 0.61\ w_p) \quad (2)$$

where $\delta\ ^{13}C_{total}$ = $\delta\ ^{13}C$ values of lipid extracted sample, w_p = protein intake from food item n, and w_c = carbohydrate intake from food item n. The value 1.5 is based on the assumption that the $\delta\ ^{13}C$ of the carbohydrate fraction is less negative than that of the protein fraction by 1.5‰. The values 0.44 and 0.61 are based on the average elemental composition of carbohydrate ($C_6H_{10}O_5$) and protein (C_5H_8ON), respectively. For foods of animal origin, this estimation was not done because of the negligible carbohydrate content. With regard to $\delta\ ^{15}N$, the measured value is considered to be equal to that of the protein fraction because protein is almost the only source of nitrogen.

TABLE 1. Proportion of measured values in the calculation of total dietary isotopic composition (‰)[1]

	Rural (Northern)	Wonie (Inland)	Ume (Riverine)	Dorogori (Coastal)
$\delta\ ^{13}C$	92	97	89	93
$\delta\ ^{15}N$	84	88	86	93

[1] (Cumulative protein intake from food items of with measured δ value)/(Total protein) × 100.

Not all of the food items observed during the surveys were sampled or measured. In this case, the protein or carbohydrate content, and $\delta\ ^{13}C$ or $\delta\ ^{15}N$ values, were estimated by the following methods:

1. Averaging the measured protein content, $\delta\ ^{13}C$, or $\delta\ ^{15}N$ of similar food items: for example, an average of the $\delta\ ^{13}C$ of papaya and mango (measured) was used for the $\delta\ ^{13}C$ of watermelon (not sampled).
2. Substitution with literature data: values used were those of Hodges et al. (1947) and the Japanese food composition table (Resources Council, 1987) for protein and carbohydrate content. $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values were adopted from Nakamura et al. (1982) and Schoeller et al. (1986).

Method 1 was preferred because in using literature data, error due to geographic variation would be added to that due to using average data from a single ecosystem. For instance, Nakamura et al. (1982) demonstrated that $\delta\ ^{13}C$ of beef varied from −13.9‰ in the United States to −23.2‰ in Germany. Therefore method 2 was used only when method 1 was not possible or inappropriate: for example, literature data on the $\delta\ ^{13}C$ value of sugar cane and corn (both C_4 plant) were used because no similar food items were sampled.

The proportion of the diet that required estimation varied from village to village and ranged from 3–16‰ of the total protein intake (Table 1).

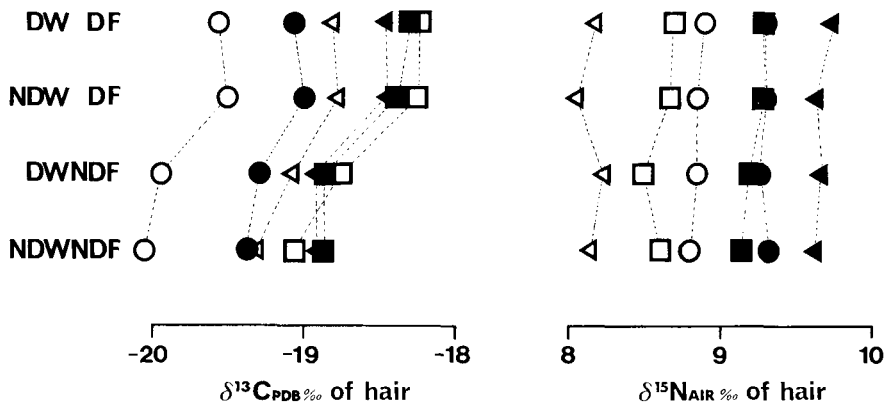

Fig. 2. Effect of washing methods on δ ^{13}C and δ ^{15}N values of Papuan hair. Hair samples of six individuals (male) from the inland village (Wonie) were used. The symbols in this figure denote the six individuals. DW DF, washed with detergent (0.4‰ polyoxyethylenerau-rylether) and solvent (chloroform + methanol: 2 + 1 and acetone); NDW DF, washed with only solvent; DWNDF, washed with only detergent; NDWNDF, not washed. Variation due to washing methods was significant ($P < 0.05$) for δ ^{13}C but not for δ ^{15}N (analysis of variance).

RESULTS AND DISCUSSION

Effect of washing on hair δ ^{13}C and δ ^{15}N

The effect of washing on δ ^{13}C and δ ^{15}N is summarized in Figure 2. Solvent-washed samples, whether washed with detergent or not, showed less negative δ ^{13}C values than other samples. No differences were found between unwashed and washed samples for δ ^{15}N. Solvent extraction removed mainly lipid, which is known to have a more negative δ ^{13}C value than protein or carbohydrate (Vogel, 1978), and which was contained in, and/or adhered to, unwashed hair. Lipid does not contain nitrogen, and this may be why there was no observed difference in δ ^{15}N between the washing methods.

Nakamura et al. (1982) did not find any differences in δ ^{13}C values of solvent-washed and unwashed hair samples from Germany, Japan, and the USA. The lack of agreement between their result and ours may be attributable to the difference in hair treatment habits between the people in these countries and the Gidra.

Chisholm (1989) stated that lipid in bone collagen and diet should be removed prior to measuring δ ^{13}C for dietary analysis because it might obscure the isotopic relationship betweem dietary protein and collagen. In this study, therefore, solvent washing without detergent (washing method 2, i.e., chloroform + methanol and acetone) was employed throughout.

δ ^{13}C and δ ^{15}N values of Gidra hair

Table 2 shows the mean hair δ ^{13}C and δ ^{15}N values of adult males from the four study villages. With regard to δ ^{13}C, the inland and coastal villages and the northern and riverine villages showed similar values. The mean δ ^{13}C values indicated that all the villagers basically depend on a C_3 plant-based food web. A minor but not negligible contribution from a C_4 plant-based or a marine food web is indicated in the coastal and inland villagers as deduced from the less negative hair δ ^{13}C values. Only the coastal villagers showed a more positive δ ^{15}N than the other three villages, where the values were similar to each other.

Nitrogen isotopic ratio in some plant foods

The δ ^{15}N values measured for six plant foods are shown in Table 3 along with protein content and δ ^{13}C values, which are taken from our previous studies (Ohtsuka et al., 1984; Yoshinaga et al., 1991).

The results show that the range of δ ^{15}N values is rather wide, viz., from −0.7 to 4.5‰, though all of these samples were from the Gidra land. This wide range may be at-

TABLE 2. Carbon and nitrogen stable isotopic composition of the hair from adult males of Gidra divided by villages

	Rual (Northern)	Wonie (Inland)	Ume (Riverine)	Dorogori (Coastal)
N	15	13	10	11
$\delta\ ^{13}C$(‰)	−20.5 ± 0.6	−19.2 ± 0.8	−21.1 ± 0.9	−18.9 ± 0.4
$\delta\ ^{15}N$(‰)	9.1 ± 0.5	8.9 ± 0.7	9.4 ± 0.6	11.3 ± 0.6

TABLE 3. Isotopic composition of six plant foods

Common name	Scientific name	Protein[1] content	$\delta\ ^{13}C$[2] (‰)	$\delta\ ^{15}N$ (‰)
Sweet potato (tuber)	*Iponea batatas*	1.1	−26.9	2.8
Elephant-foot yam (tuber)	*Amorphophallus campanulantus*	1.5	−26.8	−0.7
Banana (tuber)	*Musa* sp.	1.0[3]	−26.7	1.1
Cycad (seed)	*Cycas circinalis*	5.9	−24.1	0.3
Lotus (seed)	*Nelmubo nucifera*	6.9	−24.9	4.2
Jointfir (leaf)	*Gnetum gnemon*	4.7	−27.1	4.5

[1] Data from Ohtsuka et al. (1984) and Ohtsuka and Suzuki (1990). Unit grams/100 g edible portion.
[2] Data from Yoshinaga et al. (1991).
[3] Mean of nine samples.

TABLE 4. Weighted $\delta\ ^{13}C$ (‰) of Gidra foods and total diet based on food consumption survey

	Rual (Northern)	Wonie (Inland)	Ume (Riverine)	Dorogori (Coastal)
Plant foods				
Sago	−1.6	−1.1	−0.4	−0.1
Tuberous crops	−4.3	−8.3	−5.4	−5.9
Nuts and seeds	−4.7	−0.1	−1.1	−0.9
Other local plants	−2.1	−1.0	−1.7	−0.7
Purchased plants	−2.0	−2.1	−4.8	−7.6
C_4 or CAM plants	−0.0	−0.1	−0.1	−0.0
Others[1]	—[2]	—	—	−0.1
Animal foods				
Grass wallaby (C_4 feeder)	−2.4	−3.8	−0.1	—
Other terrestrial mammals	−1.4	−2.1	−5.7	−0.0
Birds	−0.2	−2.1	−0.3	−1.2
Other terrestrial animals	−0.3	−0.3	−0.3	—
Marine fishes	—	—	—	−2.6
Other fishes	−3.5	—	−2.5	−1.7
Other aquatic organisms	−0.1	—	−3.6	−0.5
Purchased animal	−0.0	—	−0.0	−1.3
Total (‰)	−22.7	−21.0	−25.9	−22.6

[1] Includes beverages (tea, beer, and fruit juice).
[2] No consumption was observed.

tributable to the differences in tissue sampled (e.g., leaf, tuber, or seed) as well as those between plant species.

Isotopic composition of the Gidra diet

Tables 4 and 5 show the calculated $\delta\ ^{13}C$ and $\delta\ ^{15}N$ of the Gidra's diet for each of the four villages. In these tables, weighted δ values of each food category are also presented, although the actual calculation was done on an individual food item basis, viz. $\delta\ ^{13}C$ of tuberous crops is calculated from $\delta\ ^{13}C_{taro} \times w_{taro} \times p_{taro} + \delta\ ^{13}C_{yam} \times w_{yam} \times p_{yam} + \delta\ ^{13}C_{sweet\ potato} \times w_{sweet\ potato} \times p_{sweet\ potato} + \ldots$ according to equation 1 to show the contribution of each food category to the total dietary δ value. Plant foods contribute 50–70‰ of the total dietary $\delta\ ^{13}C$. Among the plant foods, the contribution from tuberous crops (yam, taro, sweet potato, etc.) was significant in all of the villages. Considerable contributions from nuts and seeds in the northern village and purchased plant foods (rice and wheat flour) in the coastal village were noted. Other categories of plant

TABLE 5. Weighted δ ^{15}N (‰) of Gidra foods and total diet based on food consumption survey

	Rual (Northern)	Wonie (Inland)	Ume (Riverine)	Dorogori (Coastal)
Plant foods				
Sago	0.1	0.0	0.0	0.0
Tuberous crops	0.1	0.4	0.1	0.2
Nuts and seeds	0.5	0.0	0.2	0.2
Other local plants	0.1	0.0	0.1	0.0
Purchased plants	0.4	0.3	1.0	1.7
C_4 or CAM plants	0.0	0.0	0.0	0.0
Others[1]	—[2]	—	—	0.0
Animal foods				
Grass wallaby (C_4 feeder)	0.5	0.7	0.0	—
Other terrestrial mammals	0.3	0.3	1.1	0.0
Birds	0.0	0.2	0.0	0.2
Other terrestrial animals	0.1	0.0	0.1	—
Marine fishes	—	—	—	2.2
Other fishes	1.1	—	0.9	0.7
Other aquatic organisms	0.0	—	0.7	0.3
Purchased animals	0.0	—	0.0	0.7
Total (‰)	3.3	2.0	4.3	6.3

[1] Includes beverages (tea, beer, and fruit juice).
[2] No consumption was observed.

foods contribute to a similar extent in the four villages. Animal foods contribute slightly less to the total dietary δ ^{13}C value than do the plant foods. Categories of animal foods contributed different amounts in the different villages, although fish contributed a similar amount in all the villages except the inland village. The grass wallaby contributed significantly in the northern and inland villages. Contributions from other terrestrial animals (mainly pig) and other aquatic organisms (mainly freshwater clams and prawns) were significant in the riverine village. All of these are the consequence of the diversity of ecological settings of the villages, which results in differences in the availability of the various food items (Ohtsuka et al., 1985).

Contribution to the δ ^{15}N value from animal foods (local and purchased) exceeded that from local plant foods because of the more positive values of the former. Purchased plant foods and fishes, particularly marine fishes, were the main contributors to the total dietary δ ^{15}N. Lack of foods from these two categories in the inland village resulted in the least positive total dietary δ ^{15}N.

Comparison of istopic composition of diet and hair

Table 6 compares δ ^{13}C and δ ^{15}N values of hair and those of diet broken down by the villages. The difference between the values of hair and diet (diet-hair enrichment) varied from +1.8 to +4.8‰ for δ ^{13}C and +5.0 to +6.9‰ for δ ^{15}N. Table 7 lists previously reported diet-hair enrichment values. It is now accepted, from the studies listed in this table, that diet-hair enrichment for ^{13}C and ^{15}N is +1.4–2.0‰ and +4.3‰, respectively (Minagawa et al., 1986). Compared with these values, those obtained in the present study were variable and generally greater. Only the enrichments of ^{13}C in the northern and inland villages were within or close to the reported values. Enrichments of ^{13}C in the other two villages were 2–3‰ greater. On the other hand, enrichments of ^{15}N were systematically greater by approximately 1‰ than accepted values in all of the villages

TABLE 6. Comparison of hair (measured) and diet (calculated) δ ^{13}C and δ ^{15}N[1]

	Rual (Northern)	Wonie (Inland)	Ume (Riverine)	Dorogori (Coastal)
δ ^{13}C (‰)				
Hair	−20.5	−19.2	−21.1	−18.9
Diet	−22.7	−21.0	−25.9	−22.6
Enrichment	+2.2	+1.8	+4.8	+3.7
δ ^{15}N (‰)				
Hair	9.1	8.9	9.4	11.3
Diet	3.3	2.0	4.3	6.3
Enrichment	+5.8	+6.9	+5.1	+5.0

[1] Enrichment = hair-diet.

TABLE 7. Comparison of diet-hair enrichments of ^{13}C and ^{15}N (‰)

Population	N[1]	^{13}C	^{15}N	Reference
USA	15	+1.7	—	Nakamura et al. (1982)
Japan	15	+3.2	—	
Germany	15	+3.2	—	
USA	9	+1.4	+4.4	Schoeller et al. (1986)
Japan	23	+1.2	+3.8	Minagawa et al. (1986)[2]
China	7	+1.0	+4.6	
Korea	10	+1.5	+4.3	
Netherland	5	+3.0	+4.3	
Japan	42	+2.5	+4.0	Minagawa (1992)[3]
Papua New Guinea				This study
Northern	15	+2.2	+5.8	
Inland	13	+1.8	+6.9	
Riverine	10	+4.8	+5.1	
Coastal	11	+3.7	+5.0	

[1] Number of hair samples analyzed.
[2] Calculated only for the natives (e.g., Chinese living in the USA were excluded from China data).
[3] Dietary protein-hair enrichment values.

except for the inland village, where a markedly high value was obtained (6.9‰).

Possible variation caused by the process of estimating dietary isotopic composition

The first possible source of error to be considered is the representativeness of isotopic composition of foods measured in the present study. Since only one sample was analyzed for each food item (Yoshinaga et al., 1991), variation in the isotopic composition within an item might have introduced an estimation error into the dietary isotopic value. Variation in isotopic ratios within a single part of a single species of plant or animal was reported to fall in the range of several tenths to 1‰ (DeNiro and Epstein, 1978, 1981; Shearer et al., 1980; Tieszen et al., 1983; Koyama et al., 1985; Tieszen and Boutton, 1989; Mizutani et al., 1992). If the food items of the Gidra have a similar isotopic variation, so does the diet. Therefore, the probable variation in diet-hair enrichment can be estimated to be only ±1‰. This does not explain greater deviations or 2–3‰ enrichments of ^{13}C and ^{15}N.

The most probable reason for the systematic deviation to higher values can be found in the process used to estimate the dietary isotopic composition. Estimation of the δ ^{13}C value of protein fraction of a plant food, for example, might be a source of error. Although we adopted a value of 1.5‰ for the carbon isotopic difference between the carbohydrate and protein fractions according to Nakamura et al. (1982) (equation 2), this value might not be applicable in all cases. In fact, the data presented by Schoeller et al. (1986) showed that the difference between the protein and carbohydrate fractions of plant foods ranged from −1.9 to +1.5‰, with the mean being 0.3‰. When we calculated the dietary δ ^{13}C value for Gidra by using a 0.3‰ difference between the protein and carbohydrate fractions of plant foods, we obtained diet-hair enrichment values systematically decreased by approximately 0.6‰ (data not shown).

Dietary habits of the Gidra indicated by hair isotopic analysis

The representativeness of the food consumption survey data for the Gidra people's dietary habits should next be considered. To what degree the survey data represent the average of long-term (during one season and over seasons) dietary habit of the Gidra people is a crucial issue, because hair samples analyzed in the present study were not controlled for their formation period. Failure of the food consumption survey to represent an averaged long-term diet may account for the observed inconsistencies. Indeed, White (1993) found carbon isotopic variation along the shaft of mummy hair, which she attributed to seasonal dietary variation.

We estimated how much modification of the diet is needed to make the δ values of hair correspond to those of the diet (on the assumption that hair-diet enrichment val-

ues for carbon and nitrogen are 1.4–2.0‰ and 4.3‰, respectively), as follows.

Rual (northern) village. Enrichment of ^{13}C was within the range of reported values when the possible variation due to isotopic variation of foods was taken into consideration. A greater ^{15}N enrichment indicated a greater dependence on food items with higher $\delta\ ^{15}N$, such as freshwater fish or terrestrial mammals. A higher dependence on the grass wallaby (C_4 plant feeder) was not likely because this would make the ^{13}C enrichment unacceptable. If, for example, the dependence on fish (13‰ of total protein intake) doubles at the expense of plant foods, the diet-hair enrichment value of ^{13}C does not change and that of ^{15}N becomes 4.8‰, which is in good agreement with the reported value. More dependence on fish in this village has already been indicated by mercury analysis of the hair samples presently being studied (Suzuki et al., 1988).

Wonie (inland) village. The food consumption survey data are most likely to best represent the average long-term dietary habit of villagers in Wonie because food consumption surveys have been repeatedly carried out in this village (dry and wet season in 1971 [Ohtsuka, 1983]) and there were no substantial differences in the food consumption pattern, which might affect dietary isotopic composition, either between seasons or between the 1971 and 1981 surveys.

If, however, we assume that the data from the three food consumption surveys failed to represent the long-term dietary habits of the inland villagers, then the present hair data indicated that foods with high $\delta\ ^{15}N$ values significantly contributed to the protein intake of the villagers. The major food categories contributing to the protein intake of the inland villagers were C_3 plants, and C_4- and C_3-plant-feeding herbivores (Tables 4, 5). Of the foods listed during the food consumption survey in the inland village, only the monitor lizard had a high enough $\delta\ ^{15}N$ value (8.3‰, [Yoshinaga et al., 1991]) to potentially make the total dietary $\delta\ ^{15}N$ higher (thus lessening ^{15}N enrichment). If, for example, C_3 plants, C_4 plant feeders, and monitor lizard contributed equally to the protein component of the diet of the inland villagers, then the diet-hair enrichment values agreed with accepted values within ±1‰. The other potential food item is fish. Although fish is not an item consumed daily in the inland village, freshwater fish consumption was recorded during the wet season of the 1971 survey (Ohtsuka, 1983). The average isotopic composition of freshwater fish (−27.3 and 8.8‰) was similar to that of the monitor lizard; again, therefore, at least a 30‰ contribution is required to cause the reported diet-hair enrichment. However, monitor lizard contributed only 0.4‰ (1981 dry season) and fish 3.6‰ (1971 wet season [Ohtsuka, 1983]) of the total protein intake. Thus, from the standpoint of hair isotopic analysis with the assumption that the diet-hair enrichment of ^{15}N is ~4.3‰, a picture of dietary habits different from that obtained from food consumption surveys can be drawn for the inland villagers. However, the inland villagers' hair mercury levels correlated with mercury intake level estimated from the same food consumption survey data as used in the present study (Suzuki et al., 1988), indicating that the fish or reptile (both contain mercury at elevated levels) consumption of inland villagers is not much greater than revealed by the food consumption surveys.

Ume (riverine) village. The greater than reported ^{13}C and ^{15}N enrichment values indicated more dependence on mammals feeding on C_4 plants or marine organisms (both having less negative $\delta\ ^{13}C$ and more positive $\delta\ ^{15}N$) than was revealed by the food consumption survey. If, for example, protein intake from pig (17‰ of total protein intake) was halved and that from grass wallaby increased by the same proportion, then the ^{13}C enrichment decreases by ~1.5‰. Taking the possible error range in dietary $\delta\ ^{13}C$ estimation into consideration, this simple assumption makes ^{13}C enrichment acceptable in terms of the previously reported value.

Dorogori (coastal) village. The present hair isotope data indicated more dependence on marine fish than was revealed by the food consumption survey. If the contribution by marine fish to total protein intake was increased by 25‰ at the cost of plant foods,

enrichment of ^{13}C and ^{15}N decreased by 1‰ and 0.5‰, respectively, both then being close to the reported enrichment values. However, mercury analysis of hair indicated that the food consumption survey data might overestimate fish consumption by coastal villagers (Suzuki et al., 1988).

Thus, small modifications in the dietary protein sources revealed by the food consumption surveys, which seem acceptable when taking possible seasonal variation in dietary composition into account, makes diet-hair δ ^{13}C and δ ^{15}N values close to previously reported values. An exception is the inland village, where a completely different dietary habit is required to account for the ^{15}N enrichment value.

Modifying factors in the diet-hair isotopic relationship

As it has become more and more evident that diet-tissue enrichment values fluctuate because of various environmental and nutritional factors (Ambrose 1991, 1993), it is necessary to examine whether previously reported diet-hair enrichment values are applicable to all cases.

Climatic variation should possibly be considered to account for the δ ^{15}N results reported here. It is known that there is a negative correlation between rainfall and the δ ^{15}N value of plants and animals (Heaton et al., 1986; Sealy et al., 1987; Heaton, 1987; Ambrose, 1991). This observation was made in the arid savanna of Africa, so it may also hold true in the Gidra land where savanna is the dominant vegetation. Although the annual rainfall in the Gidra land reaches 2,000 mm, 80‰ of it falls in the wet season (December to May) (Ohtsuka, 1983). Drying up of small creeks in the inland village was observed during the dry season (Ohtsuka, 1983). Therefore, the possibility that climate in the dry season of the Gidra land, at least in part, contributes to the observed greater ^{15}N enrichment cannot be excluded. However, mammals and birds caught in the inland villagers' territory, as well as in other Gidra areas, did not show any greater enrichment of ^{15}N. This observation does not support the involvement of a climatic factor.

Nutritional stress is known to elevate ^{15}N enrichment (Ambrose, 1991), but this is unlikely to be the case for the Gidra because their nutrition is considered to be adequate, at least for major nutrients (Ohtsuka et al., 1985).

More importantly, Tieszen et al. (1983) demonstrated that diet-tissue enrichment of ^{13}C fluctuated according to the diet, viz., wheat (C_3 plant) or corn (C_4 plant), in experimental animals. Fluctuation of the diet-hair enrichment of ^{15}N, as well as of ^{13}C, due to the isotopic ratio of food has been noted (Minagawa et al., 1986). Since the Gidra depend on tuberous crops as a major plant protein source, the diet-tissue relationship may be different from that of modernized countries where cereals are the major source. However, if this factor modified the diet-hair isotopic relationship in the Gidra people, the effect would be common to all four villages. Therefore, the systematic deviation of enrichment to the higher values observed for both δ ^{13}C and δ ^{15}N may be, in part, explained (though the extent is not known). Thus, the greater ^{15}N enrichment observed only in the inland village still remains unexplained.

CONCLUSIONS: IMPLICATIONS FOR THE ISOTOPIC RECONSTRUCTION OF PAST DIETS

In the present study on a contemporary population, it was shown that the isotopic composition of hair samples from three villages approximated that expected from the food consumption survey data if errors in the process of estimating the dietary isotopic composition and possible temporal/seasonal variation in food consumption patterns were taken into account. This result justifies the carbon and nitrogen isotopic approach for reconstructing past human diets.

It was also found that there was an apparent discrepancy between the hair isotopic composition and the food consumption survey data in one of the four villages studied (the inland village). This could not be explained by temporal/seasonal variations in the food consumption patterns of the villagers because repeated food consumption surveys were carried out in this village and no seasonal variation was observed. This result coincided with the recent notion that diet is not the sole determinant of the isotopic

composition of animal tissue. However, none of the factors known to modify the diet-tissue isotopic relationhip, viz., climate, nutrition, and food composition, could clearly explain the observed discrepancy. Involvement of other, presently unknown, factors was thus indicated.

The existence of such modifying factors in the diet-tissue isotopic relationship has critical importance to the isotopic reconstruction of past diets because researchers have assumed that diet is the sole determinant of the isotopic composition of human tissue. It is, therefore, essential to identify the factor(s) other than diet that are involved in the case of the inland village of the Gidra reported in this paper, both to confirm the correct interpretation of past work and to ensure that future research is soundly based.

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